URINARY IL-18 AS AN EARLY BIOMARKER FOR ACUTE KIDNEY INJURY IN SNAKE BITE

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CERTIFICATE

This is to certify that the dissertation titled **"URINARY IL-18 AS A EARLY BIOMARKER FOR ACUTE KIDNEY INJURY IN SNAKE BITE"** is the bonafide original work of **Dr. JOSEPH T,** in partial fulfillment of the requirements for M.D. Branch – I (General Medicine) Examination of the Tamilnadu DR. M.G.R Medical University to be held in MARCH 2010. The Period of study was from March 2008 to March 2009.

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DECLARATION

I, **DR. JOSEPH T,** solemnly declare that dissertation titled **"URINARY IL-18 AS A EARLY BIOMARKER FOR ACUTE KIDNEY INJURY IN SNAKE BITE"** is a bonafide work done by me at Madras Medical College and Government General Hospital, Chennai, during March 2008 to March 2009 under the guidance and supervision of **Prof. A.Radhakrishnan, M.D.,** and **Prof. P.CHITRAMBALAM ,**Professors of Medicine, Madras Medical College and Government General Hospital, Chennai.

This dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (Branch – I) in General Medicine.**

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CONTENTS

INTRODUCTION

"The purpose of medicine is to prevent disease, to decrease pain and to postpone death……. Technology has to support these goals…… if not, it may not serve mankind", Dr.Timothy J Thomas (Consultant Physician, Mayo Clinic) technology.

Of all diseases known to mankind, many are preventable and most can be controlled if detected at an early stage. One such is Acute Kidney Injury (AKI).

Acute Kidney Injury (AKI) is responsible for 3-7% of hospitalisation[4] and approximately 25-30% of patients in intensive care unit[4]. The mortality rate estimates of AKI vary from 25-90%[4]. The in- hospital mortality of 40-50% and ICU mortality rate of 70-80%[4,5] (western data). Available investigation today have many times been late in detecting AKI and have caused serious delay which has caused considerable increase in mortality in patients admitted with AKI.

Several biomarkers have been thrown into limelight to be an early predictor of AKI. Neutrophil Gelatinase Associated Lipocalcin(NGAL), cystatin-C, Urinary IL – 18(U IL18), Kidney Injury Molecule (KIM-I) are some of them[1,2,8].

There has been considerable disparity in results of few studies which have used them. Some have shown to be promising and others equivocal.

UIL 18 is one such marker with varied results. Previous studies demonstrated that urinary IL-18 was raised in mouse and human AKI. But levels to predict early AKI, sensitivity and specificity and predictive values have not been clearly defined. An UIL 18 study was carried to know the utility of this biomarker in predicting AKI earlier than now. The platform to test this study was chosen to be on patients admitted with snake bite and going in for acute

kidney injury.

The goal of this study were to determine the association between UIL 18 and AKI and the reliability for them to be used as early marker in detecting AKI in comparison with pre existing surrogate markers of kidney injury like creatinine and urea.

AIMS AND OBJECTIVES

1. To assess urinary IL – 18 levels in snake bite patients.
2. To co-relate with established markers of renal failure - urea and creatinine.
3. Early detection of impending renal failure in snake bite patients.
4. To extrapolate the usefulness and applicability in other clinical situations causing AKI.

REVIEW OF LITERATURE

As mentioned before AKI contributes to 40-50% of in-hospital and 70-80% of intensive medical care unit (ICU) mortality[4] .These figures are predicted to be higher in Asian population. These figures tell us the magnitude of disease in itself.

So, in 2004 in order to bring uniformity across the globe the Acute Dialysis Quality Initiative work group set forth a definition and classification system for acute renal failure (later termed as AKI) described by the acronym RIFLE (Risk ,injury, failure, loss, end stage kidney disease).

Investigators have since applied the RIFLE system to the clinical evaluation of AKI, although it was not originally intended for that purpose. AKI research increasingly uses RIFLE. For all practical purposes AKI is defined as 50% rise in creatinine from baseline. Researchers have used different clinical settings like ICU, contrast nephropathy, diabetic nephropathy and ARDS trial to find early biomarkers to detect AKI so that considerable mortality and morbidity could be alleviated. The following table shows RIFLE classification using GFR and urine output criteria to classify AKI[2,4].

Stage	GFR Criteria	Urine Output Criteria	Probability

Risk	SCreat increased × 1.5 *or* GFR decreased >25%	UO <0.5 mL/kg/h × 6 h	High sensitivity (Risk >Injury >Failure)
Injury	SCreat increased × 2 *or* GFR decreased >50%	UO <0.5 mL/kg/h × 12 h	
Failure	SCreat increased × 3 *or* GFR decreased 75% *or* SCreat ≥4 mg/dL; acute rise ≥0.5 mg/dL	UO <0.3 mL/kg/h × 24 h (oliguria) *or* anuria × 12 h	
Loss	Persistent acute renal failure: complete loss of kidney function >4 wk		High specificity
ESKD*	Complete loss of kidney function >3 mo		

Table: RIFLE classification of AKI (adopted from enephrology)

ESKD - end-stage kidney disease; GFR - glomerular filtration rate

SCreat - serum creatinine; UO - urine output

Patients can be classified by GFR criteria and/or UO criteria.

The criteria that support the most severe classification should be used.

The superimposition of acute on chronic failure is indicated with the designation RIFLE-F $_C$; failure is present in such cases even if the increase in SCreat is less than 3-fold, provided that the new SCreat is greater than 4.0 mg/dL and results from an acute increase of at

least 0.5 mg/dL.

When the failure classification is achieved by UO criteria, the designation of RIFLE-F_O is used to denote oliguria.

The initial stage, risk, has high sensitivity; more patients will be classified in this mild category, including some who do not actually have renal failure. Progression through the increasingly severe stages of RIFLE is marked by decreasing sensitivity and increasing specificity.

With improved classification and treatment modalities, mortality due to AKI still remains high. This can be attributed to difficulty in early recognition and timely institution of treatment as existing markers of renal dysfunction show up when it has already been late in most cases. Handful of new markers have showed promise in recent times, to better existing biomarkers in this aspect .The validity of such markers can be assessed with snake bite since it forms 15% of AKI occurring in our country[10].

Although nearly all snakes with medical relevance can induce acute renal failure. In India, the incidence of AKI following Russels viper bite, the common snake bite is 13–30%[10]. Mortality due to acute kidney injury resulting from snake bite ranges from 5 –50% in primary to tertiary health set up[10]. These make snake bite an ideal clinical scenario to study the utility of proposed early renal biomarkers.

While precise figures for global snake bite epidemiology are not available, best estimates suggest that there are more than 2.5 million venomous snake bites annually, with greater than 125,000 deaths[34]. In India, a large proportion of snake bites occur when people are working barefoot in the fields, or while walking at night or early morning through fields or

along roads. In temperate countries, most snake bites occur in summer among holiday makers in the coastal regions.

. With some of the most dangerous species, such as the carpet viper, now invading cities and towns, urban dwellers are at increasing risk of fatal bites. There are more than 3000 species of snakes in the world but only about 350 are venomous[10].

Acute kidney injury (AKI) is unusual except with bites by Russells viper, E. Carinatus and members of the genera Crotalus and Bothrops. The most prevalent areas for these snakes are Asia and South America. Upto 90% of the approximately 1000 deadly snake bites occurring per annum in India and Burma are attributed to Russells viper which is also the fifth most common. cause of death In Thailand, 70% of ARF cases have been ascribed to Russells viper envenomation. In India, ARF is mostly associated with Russells viper and E. carinatus bites. The snakes of the genus Bothrops are the leading cause of venomous snake bite in South America[10].

The exact pathogenesis of ARF following snake bite in not well established. This is due to the lack of a reproducible animal model. However, a number of factors may contribute viz, bleeding, hypotension, circulatory collapse, intravascular hemolysis, disseminated intravascular coagulation, microangiopathic hemolytic anemia and also direct nephrotoxcity of the venom. Each of these factors is elucidated for better understanding of the pathogenesis and implication in AKI

Hypotension

Bleeding either into tissues or externally and loss of plasma into the bitten extremity can

produce hypotension and circulatory collapse. This is caused by venom metalloproteinases that degrade basement membrane proteins surrounding the vessel wall, leading to loss of integrity. Hemorrhagic toxins have been isolated from venom of many snakes of Viperidae and Crotalidae families. Additionally, vasodilatation and increased capillary permeability, both as a result of direct and indirect effects of venom, can aggravate the circulatory disturbances of shock. Irrespective of the cause, hypotension and circulatory collapse set in motion a chain of hemodynamic disturbances, which are known to culminate in ischemic ARF.

Intravascular hemolysis

Major factor thought to have pathogenetic significance in snake-bite-induced ARF is intravascular hemolysis. Hemolysis results from the action of phospholipase A2 which is present in almost all snake venoms and a basic protein called direct lytic factor, found only in elapid venoms. Phospholipase A2 causes hemolysis by direct hydrolysis of red cell membrane phospholipids or indirectly via the production of the strongly hemolytic lysolecithin from plasma lecithin. Evidence of intravascular hemolysis in the form of anemia, jaundice, reticulocytosis, raised plasma free hemoglobin, abnormal peripheral blood smear, and hemoglobinuria is present in about 50% of patients following bites by the Russells viper and E. carinatus. In an experimental model using male Wistar rats, severe hemolysis was shown by increased plasma LDH levels, free hemoglobin and late presence of hemolysed red blood cell casts in renal tubules after infusion of venom.

Some have even suggested that renal failure following snake bite should be considered an example of the hemolytic uremic syndrome. However, while intravascular hemolysis is

frequently observed, microangiopathic hemolysis as seen in hemolytic uremic syndrome is encountered only rarely. More over, more than 70-80% of patients with snake bite induced renal failure have only acute tubular necrosis and do not exhibit the glomerular and arteriolar changes characteristically associated with the hemolytic uremic syndrome.

Disseminated intravascular coagulation

The human haemostatic system is regulated via a number of critical interactions involving blood proteins, platelets, endothelial cells, and sub-endothelial structures. Snake venom proteins and peptides are known to activate or inactivate many of these interactions. Snake venoms, particularly those from the viper and pit viper families, contain many proteins that interact with members of the coagulation cascade and the fibrinolytic pathway. Russells viper venom (RW) contains a factor V-activating serine proteinase, which has been separated from a factor X-activating protein, also present in this venom. The enzyme (RW-V) is a single chain glycoprotein with a molecular weight of 26,100 possessing one glycosylation site near the carboxyterminus. RW-V cleaves a single peptide bond to convert factor V to factor V a (the activated clotting protein). Russells viper venom also contains a potent activator of human coagulation factor X; this enzyme has been well characterized and is designated as RW-X. Factor X activators have also been isolated from Bothrops atrox and several other snake species. Russells viper venom also activates factor IX by cleavage of a single peptide bond resulting in the formation of factor IX a.

There are several different types of prothrombin activators in snake venom. The activity of members of group I is not influenced by components of the prothrombin activator complex (factor V a, CaCI2 and phospholipid). Ecarin, from E. carinatus venom, is the most well

studied member of this group. Group II activators resemble factor X a and can cleave both peptide bonds in prothrombin, leading to active 2-chain thrombin. Their activity is strongly stimulated by phospholipids and factor V a in the presence of Ca 2. By contrast, activators in group II require only phospholipid and Calcium for the activation of prothrombin. They do not require factor Va, but appear to possess a co-factor that is tightly bound to the catalytic subunit that plays a similar role to factor Va in prothrombin activation. This class of activator is also found in Australian elapids and is represented by the high molecular weight activator from Taipan venom.

Although thrombin has many activities, the ability of some snake venom enzymes to clot fibrinogen has resulted in these enzymes being called “thrombin like”. [These are widely distributed primarily in the venom of snakes from true vipers (Bitis gabonica, Cerastes vipera) and pit vipers (Agkistrodon contortrix, Crotalus adamanteus, Bothrops atrox). Snake venom fibrinogen clotting enzymes have been classified into several groups based on the rates of release of fibrinopeptides A and B from fibrinogen.

One mechanism of the anticoagulant action of snake venom proteins is attributed to the activation of protein C. Activated protein C degrades factors V a and VIIIa and therefore, has anticoagulant activity. Major mechanism of anticoagulation involves inhibition of blood coagulation factors IX and X by a venom protein that binds to either or both. Finally, anticoagulation is also achieved through the action of snake venom phospholipases that degrade phospholipids involved in the formation of complexes critical to the activation of the coagulation pathway.

Direct-acting fibrinolytic enzymes have also been isolated from the venom of a number of north and south American snakes, including rattlesnakes and copperheads, and from elapids,

including cobras and European vipers. The venom fibrinolytic enzymes that have been characterized in detail are zinc metalloproteinases. Snake venoms also contain a number of platelet active components, including those that cause platelet aggregation and those that inhibit platelet aggregation. The final coagulation disturbance depends upon the balance among the activity of procoagulant, anticoagulant, fibrinolytic and fibrinogenolytic components of injected venom. Disseminated intravascular coagulation (DIC) is a consistent feature in patients bitten by Russells viper, E. carinatus, booms and pit vipers. The occurrence of DIC as a major hemostatic abnormality is well documented experimentally. Infusion of Russells viper or E. carinatus venom into rhesus monkeys resulted in abnormal coagulation parameters suggestive of DIC within two hours of injection of a lethal dose of the venom, but these changes first occurred from a few hours to three weeks after sub-lethal envenomation.

The presence of fibrin thrombi in the renal microvasculature and in the glomerular capillaries, and the findings of microangiopathic hemolytic anemia and thrombocytopenia in patients with cortical necrosis strongly suggest that DIC plays a major pathogenetic role in snake-bite induced cortical necrosis. Snake venom initiates a chain reaction involving the coagulation, fibrinolytic, kinin and complement systems. Venom-induced alterations lead to vascular coagulation and to deposition of fibrin thrombi in blood vessels. These changes occur in patients as well as in experimental models. Intraglomerular fibrin deposition of lesser degree has been suspected as causing acute tubular necrosis via a temporary hemodynamic alteration.

The role of the above factors in causing ARF was shown in an experimental model by Burdmann et al. They intravenously injected male Wistar rats with 0.4 mg/kg of russels viper venom and produced functional and morphological changes similar to those observed in human snake-bite induced ARF. There was an acute and significant decrease in the glomerular

filtration rate, urine output, renal plasma flow and serum fibrinogen levels. There was intravascular hemolysis, as shown by a significant decrease in hematocrit, an increase in plasma lactate dehydrogenase levels and free hemoglobin. Light and electron microscopy showed massive fibrin deposition in glomerular capillaries apart from proximal and distal tubular necrosis and red blood cell casts in renal tubules. In this model, ischemia related to glomerular coagulation and intravascular hemolysis were the most important pathogenetic factors causing a decrease in the glomerular filtration rate, although direct venom nephrotoxicity could not be excluded.

Direct nephrotoxicity

The earlier experimental studies performed on rabbits with russels venom did provide important clues to the evolution of glomerular lesions occasionally seen in human snake bite victims, but these do not seem to be relevant to patients developing renal failure, as most of them show histological changes of acute tubular or cortical necrosis. Urinary beta-N acetyl showed considerable change in patients bitten by Russells viper, without DIC, indicating a direct toxic effect of venom on the kidney.

In a study, the administration of a lethal dose of Russells viper or E. carinatus venom to rhesus monkeys, resulted in hemorrhages in the kidneys and other organs in all animals, and mild acute tubular necrosis in 20% of animals, within 24 hrs of envenomation. After a sublethal venom dose, however, more than 50% of animals developed acute tubular necrosis, and fibrin thrombi were demonstrable in 50-75% of glomeruli. The histological findings and the coagulation abnormalities observed in these animals were similar to those seen in human

victims of snake bite. The strongest evidence supporting direct nephrotoxcity is a dose-dependent decrease in inulin clearance and an increase in fractional excretion of sodium in the isolated perfused rat kidney, following russels viper envenomation.

On morphological analysis, the most prominent structural lesions were observed in the renal cortex. Extensive damage and loss of glomerular epithelial cells and endothelium was detected with only the basement membrane remaining. Ballooning and even rupture of glomerular capillaries could be seen. Major prominent feature of russels viper venom action on renal cortex, and likewise on all other renal zones, concerned vessels with muscular walls (arteries, veins, arterioles, venules).The venom led to complete lysis of vascular smooth muscle cells leaving behind only the basement membrane. Varying degrees of epithelial injury occurred in all tubular segments In addition myoglobinuria, sepsis, and hypersensitivity to venomous or anti-venomous protein may also contribute towards renal failure. Crescentic nephritis in patients bitten by puff adder snake has been attributed to hypersensitivity to antisnake venom. Myoglobinuria generally occurs following sea snake envenomation, which results in necrosis of striated muscles and muscular paralysis.

In patients with ARF, oliguria often develops rapidly within the first 48 hrs, but may be delayed till 3-4 days after the bite. Some patients become anuric, whereas occasional patients remain non-oliguric. Urine may show gross or microscopic hematuria. Some patients complain of pain in the renal angle preceding oliguria, which may be a useful clue to impending renal failure. Jaundice and hemoglobinuria resulting from intravascular hemolysis are not infrequent following Russells viper or E. carinatus bites and have been reported from India and Sri Lanka. Daily urine protein concentration may exceed one gram, and erythrocyte casts may also be seen. Hypertension has been infrequently recorded after both viper and sea snake bites. The

mortality of patients with renal failure varies with the nature of the renal lesion. Although only 16% of those with acute tubular necrosis, in whom uremia was controlled with dialysis, died, as many as 80% of those with cortical necrosis had a fatal outcome [10].

Renal Histology in snake bites

Renal histology shows predominantly either acute tubular or cortical necrosis. A number of glomerular changes have been described but their significance is not known.

1.Acute Tubular Necrosis

Acute tubular necrosis is the predominant lesion seen in 70-80% of patients with ARF. On light microscopy, the tubules appear dilated and lined by flattened epithelium. Severe cases exhibit cell necrosis and desquamation of necrotic cells from the basement membrane. Hyaline, granular or, pigment casts are seen in tubular lumina. Varying degrees of interstitial edema, hemorrhage, and inflammatory cell infiltration are present. Later biopsies reveal regenerating tubular epithelium. Intrarenal blood vessels are usually unaffected. On ultrastructural examination, proximal tubules show dense intra-cytoplasmic bodies representing degenerating organelles or protein resorption droplets. Small areas of basement membrane are denuded. Distal tubular cells have a dilated endoplasmic reticulum and many degenerating organelles. Apoptosis is a prominent feature in the distal tubules, indicating a high cell turnover. In the interstitium, fibroblasts appear active, with increased numbers of organelles and cytoplasmic processes. Mast cells and eosinophils show both granulated and partially degranulated forms.

Although the blood vessels appear normal under light microscopy, ultrastructural abnormalities are notable in both large and small caliber vessels. Medullary vessels are severely affected, with markedly swollen, focally necrotic, endothelial cells obliterating the

lumen. Smooth-muscle cells show cytoplasmic vacuoles, which are empty or are filled with granular material. The severe vascular lesions, distal tubular apoptosis, and presence of mast cells, eosinophils, and active fibroblasts in the interstitium are features that have not been observed in acute tubular necrosis from other causes.

2.Acute Cortical Necrosis

Bilateral diffuse or patchy cortical necrosis has been observed following bites by E. carinatus. Cortical necrosis appears to be more common among Indian patients than among patients in other south-asian regions, for unknown reasons. The presence of fibrin thrombi in the arterioles is a prominent feature in these patients. A narrow subcapsular rim of cortex often escapes necrosis. The area underlying this, however, shows necrosis of glomerular as well as tubular elements. The necrotic zone is often bordered by an area of hyperemia and leukocytic infiltration. Calcification of necrotic areas may occur at a later stage. Varying numbers of glomeruli are spared in patients with patchy cortical necrosis. With healing, fibroblastic proliferation and organization of thrombi are seen.

3.Glomerular Lesions

Whether or not specific glomerular lesions really occur is still controversial patients. diffuse glomerular hypercellularity (ascribed to marked mesangial proliferation), ballooning of capillaries, endothelial swelling, mesangiolysis and splitting of the glomerular basement membrane; however, the significance of these is difficult to ascertain. Immunofluorescence microscopy showed 1gM, C3, and fibrin deposits. In occasional instances, a diffuse and intense mononuclear cell infiltrate has been noted in the interstitium, suggesting the occurrence of an acute interstitial nephritis.

Various factors contribute to the pathogenesis of acute renal failure in snake bite,

namely bleeding, hypotension, circulatory failure, intravascular hemolysis, disseminated intravascular coagulation, microangiopathic hemolytic anemia and more importantly direct nephrotoxicity of the venom.

Renal histology shows that the predominant lesion is acute tubular neurons (ATN) in 70 – 80% with mortality rate of 16% and those with cortical necrosis, the mortality rate was found to be as high as 60%.

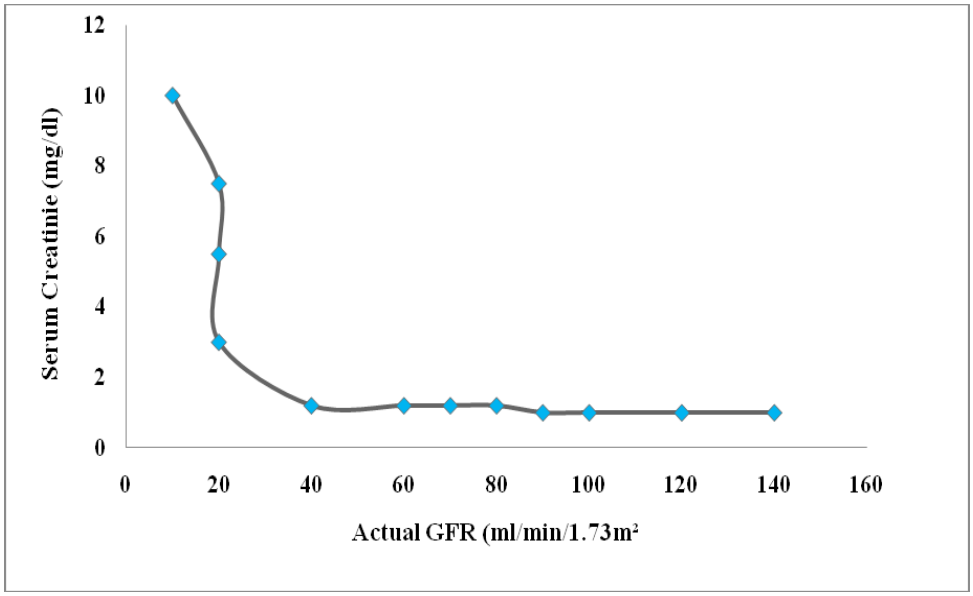
Mortality due to acute kidney injury resulting from snake bite ranges from 5 –50% in primary to tertiary health set up[10]. Major reason for this alarming number is late recognition of developing AKI and delayed referral to tertiary set up with renal replacement facilities

Animal models and human studies strongly suggest that early treatment is the key to preventing AKI and halting the progression of disease. Existing biomarkers are found to be inadequate for earlier detection and timely prevention or intervention (fluid replacement as hypotension plays a major role in AKI due to snake bite or renal replacement therapy).

Urea and Creatinine : Need for another marker?

The loss of kidney function in acute renal failure is most easily detected by measurement of the serum creatinine, which is used to estimate the glomerular filtration rate (GFR).

A low serum creatinine concentration may be misleading in patients with reduced muscle mass. In addition, serum creatinine is somewhat insensitive index of renal function, especially in early and late stages of renal disorder.



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Graph[4]: Relationship between serum creatinine and actual values of eGFR in patients with renal failure. Normal value of serum creatinine is indicated by dashed line.

Above graph shows the relative insensitivity of creatinine when GFR falls

140ml/min/1.73m^2 to 40ml/min/1.73m^2 .This depicts creatinine is slow to rise even when there is considerable fall in GFR .

Two mechanisms account for relative insensitivity of serum creatinine and urea as an indication of acute renal injury. As part of adaptation of the kidney to renal injury, uninjured nephrons undergo hypertrophy and hyper filtration. Thus, total GFR and serum creatinine and urea remain relatively normal despite a decrease in functioning nephrons[2,3,6,9,12].

Another potential problem is that tubular secretion of creatinine, which normally contributes little to overall creatinine clearance, increases progressively as renal disease worsens.

Thus, both serum creatinine concentration and the creatinine clearance becomes increasingly unreliable as estimates of GFR in patients with early renal disease.

Finally, certain circumstances are associated with spurious elevation of serum creatinine independent of changes in GFR. Rhabdomyolysis / ingestion of loaded meat, certain drugs, ketoacidosis, and acrobatics can be detected to have raised creatinine due to protein catabolism leading to false elevation.

Serum creatinine has failed as a reliable marker of renal dysfunction for various reasons – many non renal factors cause changes in creatinine levels, its increase is not directly related due to tubular injury but an effect of reduced GFR in AKI. There is also a delay in the detectable increase in serum creatinine that is sometimes marked by intravenous fluid administration and many a time this becomes too late which is reflected in the morbidity and mortality rate of AKI using urea and creatinine in detecting AKI.

So, better biomarkers are needed to spot AKI early and prevent progression.

EMERGING BIOMARKERS — Although the serum creatinine is widely used in diagnosing the presence of acute renal failure, it is a poor biomarker for this process principally due to its inability to help diagnose early acute renal failure and complete inability to help differentiate among its various causes [21] . As an example, the rise in serum creatinine is slow following the onset of acute renal failure. By the time a change is observed in the serum creatinine, a critical therapeutic window may have been missed, particularly among those with ATN.

Thus, different urinary and serum proteins have been intensively investigated as possible biomarkers for the early diagnosis of ATN. There are promising candidate biomarkers

with the ability to detect an early and graded increase in tubular epithelial cell injury and to distinguish prerenal disease from acute tubular necrosis [21,22.]

Neutrophil Gelatinase Associated Lipocalcin(NGAL), cystatin-C, Urinary IL – 18(UIL18), Kidney Injury Molecule (KIM-I) are some of them[1,2,8].

Urinary and plasma levels of NGAL were evaluated in a pilot study of 71 children undergoing cardiopulmonary bypass to identify very early markers for ATN [33] . Urinary NGAL levels obtained at two hours following procedure correlated with severity and duration of AKI, length of stay, requirement for dialysis, and death. Urinary kidney injury molecule-1 — Kidney injury molecule-1 (KIM-1) is a type 1 transmembrane glycoprotein that shows marked upregulation in the proximal tubule with AKI [39] ;the ectodomain is shed and can be measured in urine by immunoassay. Kim-1 has been tested in a number of small cohorts [19,40] .

Serum and Urinary IL-18 is also shown to be markedly raised in AKI in various cross sectional studies. Previous studies have shown sensitivity and specificity of >90% in diagnosing AKI[11,13].

IL-18 is one of the numerous pro-inflammatory cytokines produced in the body. IL-18 belongs to IL-1, super family of cytokines. The activities of IL-18 appear to species – specific. An important function of IL-18 is the regulation of functionally distinct subsets of T-helper cells required for cell mediated immune responses. IL-18 functions as a growth and differentiation of TH1 cells[9,13,24].

It is produced during the acute immune response by macrophages and immature dendritic cells. It is also produced by epithelial lining of lungs and distal and proximal tubules of kidney[24]. This fact is responsible for acute rise of urinary IL18 seen in tubular necrosis.

Urinary interleukin 18 has been found to be a useful tool in various experimental studies. Serum and urinary levels are raised in many clinical settings like pulmonary sarcoidosis, idiopathic pulmonary fibrosis, multiple sclerosis, closed head injury, crohns disease, diabetic nephropathy, coronary artery disease[22]. Its elevation is also correlated with increased carotid intimal medial thickness[24]. Many of these depict IL18 to be a surrogate marker of infection and inflammation.[24]

Urinary Interleukin-18 may be involved in sleep disorders in end-stage renal disease patients Higher plasma interleukin-18 levels are associated with poorer quality of sleep in peritonealdialysis patients[24]. Whether a cause-and-effect relationship exists between interleukin-18 and quality of sleep are under further investigation[27] Increased serum and urine IL-18 levels were observed during relapse of idiopathic nephrotic syndrome. These findings indicate the association between the active phase of idiopathic nephrotic syndrome and the levels of IL-18 and can suggest the role of this cytokine in this syndrome. Serum levels of IL-l8 might be a predictor of progression of diabetic nephropathy as well as cardiovascular diseases.

Urinary IL-18 levels start increasing within 6 –12 hours of kidney assault, much in advance to in blood urea nitrogen (BUN) and creatinine concentration[1,5,24]. In another prospective study with intensive care patients, the urine IL-18>100 pg/ml was predictive of AKI developing within 24 hours[13,24].

In a country like ours, where mortality from acute kidney injury has been high, early recognition and prompt intervention will be required to save lives. Last, intensive medical resources should be made available in all centres to help patients developing AKI.

MATERIALS AND METHODS

SETTING

In-patients

Toxicology ward

Institute of internal medicine

Madras Medical College and Government General Hospital

Chennai-600 003

ETHICAL COMMITTEE APPROVAL

Obtained

STUDY DESIGN

Single centre, description, cross sectional, non-interventional study

PERIOD OF STUDY

March 2008 – March 2009

SAMPLE SIZE

80 patients

SELECTION OF STUDY PATIENTS

INCLUSION CRITERIA

Patients admitted in Toxicology with snake bite.

EXCLUSION CRITERIA

1. Patients with existing renal parenchymal disease.
2. Other conditions predisposing to AKI such as diabetes mellitus, hypertension, sepsis, shock and cardiovascular disease.
3. Conditions associated with raised IL-18 levels- cellulites, crohn's disease, acute pancreatitis, obesity, multiple sclerosis, pulmonary sarcoidosis, raised ICT, cerebrovascular events and presence of any infection or inflammation.
4. Patients with serum creatinine > 1.2 mgs/dl and those who received nephrotoxic agents

METHODOLOGY

The study was conducted in the toxicology wards in the Institute of Internal Medicine, Madras Medical College and Government General Hospital, Chennai.

Patients admitted with snake bite were asked to collect 5 ml of midstream urine at 12, 24, and 48 hours after bite. Their daily urine output was also charted. Urine bags of catheterized patients were measured and emptied one hour prior to collection to make collection of sample uniform and without much alteration due to concentration of urine. Of the original 104 patients, 80 were enrolled as some were dropped as they could not be relied on the exact time of bite and others could not collect specimen at stipulated time.

Samples were collected in a sterile container and freezed until they were thawed and centrifuged at 4000 rpm at 4°C for 10 minutes. The supernatant was aliquotted equally into cryovials and freezed under -20°C until UIL-18 was measured using Human IL-18 (ray bio,Medical and Biologic laboratory, Nagoya, Japan) kit. This detects the mature form of IL-18. The cross reactivity of pro-IL18 is extremely low. The co-efficient of inter and intra assay for the kit ranges from 5 – 10%.

Assay Procedure

1. Bring samples and reagents to room temperature.
2. Prepare all reagents, samples and standard as instructed.
3. Add 100 micro liter standard or sample to each wall. Incubate 2.5 hours at room

temperature or over night at 4°C.

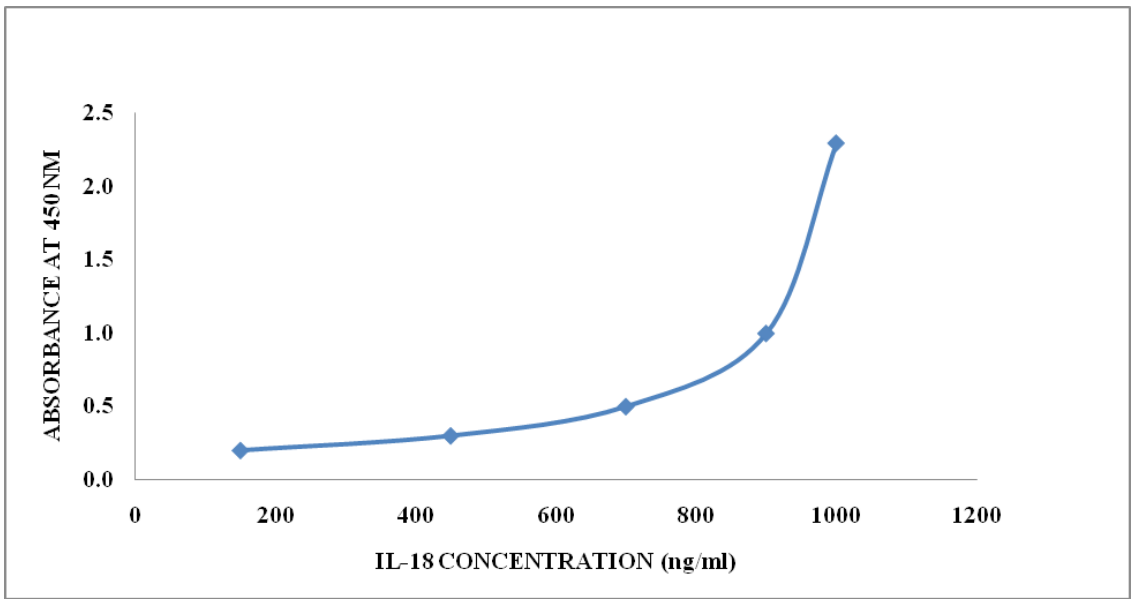
4. Add 100 micro liter prepared biotin antibody to each wall. Incubate 1 hour at room temperature.
5. Add 100 micro liter of prepared streptavidin solution. Incubate 45 minutes at room temperature.
6. Add 100 micro liter one step substrate reagent to each wall. Incubate 30 minutes at room temperature.
7. Add 50 micro liter stop solution to each wall.
8. Read at 450 nm immediately.

The standard concentration was diluted to 500, 250, 125, 100, 50.

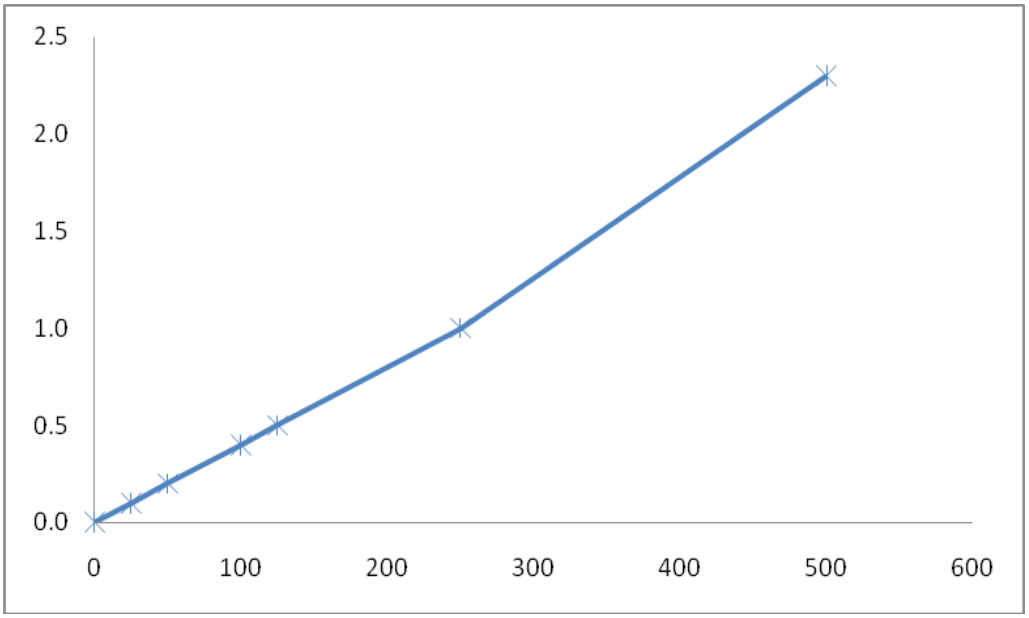
CALCULATION OF RESULTS

Samples are read using ELISA reader in biochemistry department. Calculate the mean absorbance for each set of standards, controls and samples and subtract the average zero standard optical density. Plot the standard curve by using sigma plot software, with standard concentration on the X-axis and absorbance on the Y-axis. Draw the best fit straight line through the standard points.

STANDARD CURVE FROM HUMAN IL-18 ASSAY KIT.



TEST CURVE FROM OUR DILUTED STANDARDS



All samples are read at 450 nm and optical density obtained is converted into pg/ml

through a computer fed program.

The data thus obtained are charted with help of Microsoft XP and statistical analysis using statistical package for social sciences (SPSS 12).

RESULTS

Eighty patients who presented with snake bite at the Toxicology Department of the Government General Hospital, Chennai, India, between March 2008 and March 2009 were enrolled in the study, based on the inclusion and exclusion criteria described earlier (see Patients and Methods). In each of the patients, the serum creatinine (mg/dL), blood urea (mg/dL) and urinary IL-18 (pg/ml) concentrations were evaluated at 12 hours, 24 hours and 48 hours after admission.

To facilitate analysis of the data, the 80 patients were divided into 4 groups:

a) **Group I (39 patients):**
Patients without acute kidney injury (AKI) and with urinary IL-18 levels below100 pg/ml.

b) **Group II (4 patients):**
Patients without AKI and with urinary IL- 18 levels at or above 100 pg/ml.

c) **Group III(16 patients):**
Patients with AKI and with urinary IL- 18 levels below 100 pg/ml.

d) **Group IV(21 patients):**
Patients with AKI and with urinary IL- 18 levels at or above100 pg/ml.

DIFFERENT GROUPS

TABLE 1

EVALUATION OF SERUM CREATININE LEVELS

Hours after admission	Group I(n=39)	Group II (n=4)	Group III (n=16)	Group IV (n=21)
12 hrs	0.74 ± 0.16	0.70 ± 0.26	0.83 ± 0.27	0.76 ± 0.18

24 hrs	0.74 ± 0 .18	0.90 ±0.26	1.14 ± 0.31 [a* d†]	0.82 ± 0.23 [c*]
48 hrs	0.78 ± 0.19	0.90 ± 0.26	2.09 ± 0.36 [a* b* d* e*]	2.35 ± 0.63 [a* b* c‡ d* e*]

At 12 hours, there were no significant differences between the mean serum creatinine levels in patients in groups I, II, III and IV, with the values being within the normally accepted range.

At 24 hours, the mean serum creatinine level in group III patients was significantly higher ($P<0.001$) than that in group I patients and significantly higher ($P<0.001$) than that in group IV patients, but still within the normally accepted range.

At 48 hours, the mean serum creatinine levels in patients in groups III and IV were significantly higher ($P< 0.001$ and $P<0.001$, respectively) than that in group I patients; similarly, the mean serum creatinine levels in patients in groups III and IV were significantly higher ($P< 0.001$ and $P<0.001$, respectively) than that in group II patients. In addition, the mean serum creatinine level in group IV patients was significantly higher ($P< 0.05$) than that in group III patients. The mean serum creatinine levels in group III and group IV patients at 48 hours were higher than the upper limit of the normally accepted range.

In group I patients, there were no significant differences between the mean serum creatinine levels at 12 hours, 24 hours and 48 hours, and the values were within normal limits. Similarly, in group II patients, there were no significant differences between the mean serum creatinine levels measured at 12 hours, 24 hours and 48 hours, and the values were within normal limits.

In group III patients, the mean serum creatinine level at 24 hours was significantly higher ($P<0.01$) than that at 12 hours, but was still within normal limits. However, at 48 hours, the mean serum creatinine level was significantly higher than that at 12 hours ($P<0.001$) and

that at 24 hours (P<0.001) and was higher than the upper limit of the normally accepted range.

In group IV patients, the mean serum creatinine level at 24 hours was essentially similar to that at 12 hours, and within normal limits. However, at 48 hours, the mean serum creatinine level was significantly higher than that at 12 hours (P<0.001) and that at 24 hours (P<0.001) and was higher than the upper limit of the normally accepted range.

Values are expressed as mean ± SD.
Statistical analyses (*, †and ‡ represent $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively).

[a] Group I vs. groups II, III, IV values

[b] Group II vs. groups III, IV values

[c] Group III vs. group IV values

[d] 12 hours vs. 24, 48 hours

[e] 24 hours vs. 48 hours

a) Group I: Patients without acute kidney injury (AKI) and with urinary IL- 18 levels below 100 pg/ml;
b) Group II: patients without AKI and with urinary IL- 18 levels at or above 100 pg/ml;
c) Group III: patients with AKI and with urinary IL- 18 levels below 100 pg/ml;
d) Group IV: patients with AKI and with urinary IL- 18 levels at or above 100 pg/ml

2. Evaluation of blood urea levels (Table 2)

Table 2.
Mean blood urea levels (mg/dL) in study patients

Hours after admission	Group I (n=39)	Group II (n=4)	Group III (n=16)	Group IV (n=21)
12 hrs	29.44 ± 6.50	29.50 ± 7.72	35.25 ± 8.73 [a†]	28.33 ± 6.19 [c†]
24 hrs	31.57 ± 5.81	30.50 ± 5.74	41.31 ± 5.78 [a‡]	36.77 ± 23.45 [d‡]
48 hrs	33.03 ± 5.88	34.50 ± 4.12	58.06 ± 7.35 [a* b* d* e*]	67.95 ± 21.19 [a* b* c‡ d* e*]

At 12 hours and 24 hours, there were no significant differences between the mean blood urea levels in patients in groups I, II and IV, with the values being within the normally accepted range. In group III patients, the mean blood urea levels were significantly higher than those in group I patients at 12 hours ($P<0.01$) and 24 hours ($P<0.05$) and was also significantly higher ($P<0.01$) than the level in group IV patients at 12 hours, but the values were within the normally accepted range.

At 48 hours, the mean blood urea levels in patients in groups III and IV were significantly higher ($P< 0.001$ and $P<0.001$, respectively) than that in group I patients; similarly, the mean blood urea levels in patients in groups III and IV were significantly higher ($P< 0.001$ and $P<0.001$, respectively) than that in group II patients. In addition, the mean blood urea level in group IV patients was significantly higher ($P< 0.05$) than that in group III patients. The mean blood urea levels in group III and group IV patients at 48 hours were higher than the upper limit of the normally accepted range.

In group I patients, there were no significant differences between the mean blood urea levels at 12 hours, 24 hours and 48 hours, and the values were within normal limits. Similarly, in group II patients, there were no significant differences between the mean blood urea levels measured at 12 hours, 24 hours and 48 hours, and the values were within normal limits.

In group III patients, the mean blood urea level at 24 hours was not significantly different from that at 12 hours, and the levels were within normal limits. However, at 48 hours, the mean blood urea level was significantly higher than that at 12 hours ($P<0.001$) and that at 24 hours ($P<0.001$) and was higher than the upper limit of the normally accepted range.

In group IV patients, the mean blood urea level at 24 hours was significantly higher ($P <$

0.05) than that at 12 hours, but was within normal limits. However, at 48 hours, the mean blood urea level was significantly higher than that at 12 hours (P<0.001) and that at 24 hours (P<0.001) and was higher than the upper limit of the normally accepted range.

Values are expressed as mean ± SD.

Statistical analyses (*, †and ‡represent $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively)

[a] Group I vs. groups II, III, IV values

[b] Group II vs. groups III, IV values

[c] Group III vs. group IV values

[d] 12 hours vs. 24, 48 hours

[e] 24 hours vs. 48 hours

a) Group I: Patients without acute kidney injury (AKI) and with urinary IL- 18 levels below 100 pg/ml;
b) Group II: patients without AKI and with urinary IL- 18 levels at or above 100 pg/ml;
c) Group III: patients with AKI and with urinary IL- 18 levels below 100 pg/ml;
d) Group IV: patients with AKI and with urinary IL- 18 levels at or above 100 pg/ml.

3. Evaluation of urinary IL-18 levels (Table 3)

Table 3
Mean urinary concentrations of IL-18 in study patients

Hours After admission	Mean levels (pg/ml) in group I (n=39)	Mean levels (pg/ml) in group II (n=4)	Mean levels (pg/ml) in group III (n=16)	Mean levels (pg/ml) in group IV (n=21)
12 hrs	18.47 ± 10.43	21.05 ± 17.12	27.34 ± 22.83	21.00 ± 16.10
24 hrs	19.57 ± 9.95	119.35 ± 17.94 a* d*	49.54 ± 21.76 a* b* d*	114.70 ± 12.33 a* c* d*
48 hrs	23.65 ± 11.93	142.70 ± 53.15 a* d* e‡	82.22 ± 8.96 a* b* d* e*	147.55 ± 28.33 a* c* d* e*

At 12 hours, there were no significant differences between the mean urinary concentrations of IL-18 in patients in groups I, II, III and IV, and the levels ranged from 18.47 ± 10.43 to 27.34 ± 22.83 pg/ml (Table 3).

At 24 hours, the mean urinary concentrations of IL-18 in patients in groups II, III and IV were all significantly higher ($P<0.001$, $P<0.001$ and $P<0.001$, respectively) than the mean concentration in group I patients. Moreover, the mean urinary concentration of IL-18 in group III patients, although significantly higher ($P<0.001$) than that in group I patients, was significantly lower than that in group II patients ($P<0.001$) and that in group IV patients ($P<0.001$). The mean concentrations ranged from 49.54 ± 21.76 to 119.35 ± 17.94 pg/ml in patients in groups II, III and IV.

At 48 hours, the mean urinary concentrations of IL-18 in patients in groups II, III and IV were all significantly higher ($P<0.001$, $P<0.001$ and $P<0.001$, respectively) than the mean concentration in group I patients. Moreover, the mean urinary concentration of IL-18 in group III patients, although significantly higher ($P<0.001$) than that in group I patients, was significantly lower than that in group II patients ($P<0.001$) and that in group IV patients

(P<0.001). The mean concentrations ranged from 82.22 ± 8.96 to 147.55 ± 28.33 pg/ml in patients in groups II, III and IV.

In group I patients, the mean urinary concentrations of IL-18 at 24 hours and at 48 hours were not significantly different from that at 12 hours. In group II patients, the mean urinary concentration of IL-18 at 24 hours was significantly higher (P<0.001) than that at 12 hours, while the mean IL-18 concentration at 48 hours was significantly higher than that at 12 hours (P<0.001) and that at 24 hours (P<0.05). In group III patients, the mean urinary concentration of IL-18 at 24 hours was significantly higher (P<0.001) than that at 12 hours, while the mean IL-18 concentration at 48 hours was significantly higher than that at 12 hours (P<0.001) and that at 24 hours (P<0.001). In group IV patients, the mean urinary concentration of IL-18 at 24 hours was significantly higher (P<0.001) than that at 12 hours, while the mean IL-18 concentration at 48 hours was significantly higher than that at 12 hours (P<0.001) and that at 24 hours (P<0.001).

Values are expressed as mean ± SD.

Statistical analyses (*, †and ‡ represent $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively)

[a] Group I vs. groups II, III, IV values

[b] Group II vs. groups III, IV values

[c] Group III vs. group IV values

[d] 12 hours vs. 24, 48 hours

[e] 24 hours vs. 48 hours

a) Group I (39 patients): patients without acute kidney injury (AKI) and with urinary IL-18 levels below 100 pg/ml;
b) Group II (4 patients): patients without AKI and with urinary IL- 18 levels at or above

100 pg/ml;

c) Group III(16 patients): patients with AKI and with urinary IL- 18 levels below 100 pg/ml;
d) Group IV(21 patients): patients with AKI and with urinary IL- 18 levels at or above 100 pg/ml

4. Evaluation of a urinary IL-18 concentration of ≥100 pg/ml at 24 hours as a diagnostic aid for acute kidney injury (table 4)

Table 4

Urinary IL-18 level	AKI Present	AKI not Present	Total
≥100 pg/ml	**21**	**4**	**25**
<100 pg/ml	**16**	**39**	**55**
Total	**37**	**43**	**80**

According to Parikh et al, a urinary IL-18 concentration of ≥100 pg/ml at 24 hours can be used to predict the occurrence of acute kidney injury. This aspect was examined in the present study by constructing a 2 X 2 table and then calculating important parameters. The following results were obtained: sensitivity of 56.76 % , specificity of 90.70 %, predictive value of a positive test of 86 %, predictive value of a negative test of 71 %, false-positive rate of 5 %, false-negative rate of 20%.

PARAMETERS

a) Sensitivity (21/37 x 100) = 56.76 %

b) Specificity (39/43 x 100) = 90.70 %

c) Predictive value of positive test (21/25 x 100) = 86 %

d) Predictive value of negative test (39/55 x 100) = 71 %

e) False-positive rate (4/80 x 100) = 5 %

f) False-negative rate (6/80 x 100) = 20 %

5. Evaluation of a urinary IL-18 concentration of $\geq$ 40 pg/ml at 24 hours as a diagnostic aid for acute kidney injury

Table 5

Urinary IL-18 level	AKI Present	AKI not Present	Total
≥40 pg/ml	**32**	**7**	**39**
<40 pg/ml	**5**	**36**	**41**
TOTAL	**37**	**43**	**80**

In an attempt to improve the sensitivity of the screening procedure using urinary IL-18 concentrations without marked loss of specificity, and to reduce the false-negative rate without a marked increase in the false-positive rate, another approach was taken. The normal range of urinary IL-18 at 24 hours was calculated (based on the values obtained in group I patients at 24 hours in the present study).

Mean concentration of urinary IL-18 at 24 hours in 39 normal (Group I) patients:
= 19.57 $\pm$ 9.95 pg/ml .
Normal range (95.45% accuracy) = Mean value $\pm$ 2 SD
= 19.57 $\pm$ (2 x 9.95)
= 19.57 $\pm$ 19.9
= 39.47 pg/ml
Accordingly, a concentration of $\geq$ 40 pg/ml at 24 hours was taken as a `break-point'

concentration to predict the occurrence of acute kidney injury.

When this value was taken, the following results were obtained:

a) Sensitivity = 86.48 %

b) Specificity = 83.72 %

c) Predictive value of positive test = 82.05 %

d) Predictive value of negative test = 87.8 %

e) False-positive rate = 8.75 %

f) False-negative rate = 6.25 %

PARAMETERS

a) Sensitivity (32/37 x 100) = 86.48 %

b) Specificity (36/43 x 100) = 83.72 %

c) Predictive value of positive test (32 / 39 x 100) = 82.05 %

d) Predictive value of negative test (36 / 41 x 100) = 87.8 %

e) False-positive rate (7/80 x 100) = 8.75 %

f) False-negative rate (5/80 x 100) = 6.25 %

Statistical analysis

The values are expressed as mean ± standard deviation (SD). Differences between groups were assessed by one-way analysis of variance (ANOVA) using SPSS software package for Windows (Version 11.5; SPSS Inc., Chicago, IL, USA). Post hoc testing was performed for intergroup comparisons using the least significance difference (LSD) test. Values of $P <$ 0.001, 0.01 and 0.05 have been denoted by distinct symbols in the tables.

DISCUSSION

AKI is defined by acute changes in serum creatinine, which for many reasons mentioned before is a late and non-specific marker of AKI. AKI is emerging as a public health problem since epidemiologic studies reveal that mild increase in serum creatinine of hospitalized patients are associated with increased mortality and morbidity[6]. Several urinary biomarkers have been identified as potential candidates for AKI detection prior to serum creatinine rise.

Animal studies show that intra renal IL-18 production is involved in the pathophysiology of ischemic AKI independent of neutrophil[8,29,32,35]. Human studies revealed that UIL-18 concentrations rise 24 – 48 hours prior to AKI in two meta-analytic study in ICU set up. Reports were not promising in few studies conducted in Japan in predicting contrast induced nephropathy.

Parikh et al reported the results of a nested case control study analyzing the sequential urine samples from patients enrolled in ARDS trials. They found that UIL18 levels >100 pg/ml were predictive of AKI developing within 2 days[5,24,29].

Earlier in 2005, neutrophil gelatinase associated lipocalcin (NGAL) was identified as a highly sensitive and specific marker of AKI following cardiac surgery in pediatric patients.

The discriminatory capacity of biomarkers is based on data from trials that used different inclusion and exclusion criteria and different definitions of AKI, making comparison difficult.

The current study is the first to:

1. Systematically examine the relationship between UIL18 and AKI comparing with urea and creatinine in snake bite patients.

2. To examine the impact of UIL18 utility as AKI biomarker.

The range of urinary IL-18 in different studies ranges from 10 – 25 pg/ml and serum level of 15 – 70 pg/ml[12,13]. According to Parikh et al in ARDS trial, elevation in urinary IL-18 >100 pg/ml at 24 hours predicted AKI 2 days prior to the kidney (sensitivity 95%, specificity 80%) injury manifesting as creatinine and BUN rise[29,32,34].

In the present study, mean concentration of urinary IL-18 at 24 hours in 39 normal (group I) patients was found to be 19.57 ± 9.95 pg/ml. So, the normal range in the population would be from 0 – 40 pg/ml (0.57 – 39.47 pg/ml by Mean ± 2 SD).

The following results were obtained on using 100 pg/ml as cut-off to predict AKI developing within 24 hours – sensitivity of 56.76%, specificity of 90.7%. The predictive value of a positive and negative test was around 86% and 76% respectively.

Accordingly, when 40 pg/ml, the upper limit of normal range was taken as "break-point" concentration to predict the occurrence of acute kidney injury, the sensitivity increased to 86.48% with a mild drop of specificity from 90.7 to 83.7%. The predictive value of positive and negative test remained at 82.05% and 87.5% respectively.

The most important goal for studying AKI biomarkers is AKI detection prior to serum creatinine rise. On comparing the mean tables of urea, creatinine (Tables 1, 2 and 3) and UIL18, it is seen that UIL18 rises atleast 24 hours prior to rise in BUN and creatinine if 40 mg/ml is taken as a reference point on contrary to 48 hrs according to Parikh et al.

Unfortunately our sample study was not large enough to categorize and differentiate patients with fluid respective elevation in serum creatinine (pre-renal) from renal injury as seen with acute tubular necrosis.

Snake bite above caused AKI by various mechanism mentioned previously. The most common among them was direct tubular injury. Some in group II did have a rise in UIL18, but did not progress to AKI as per creatinine levels. This is due to minimal insult from toxin of snake bite which got aborted early and this is also the reason for positive predictive and negative predictive value to be perched at mid 80's as UIL18 also picked up patients who develop kidney insult even when it did not show in creatinine levels later.

The observation adds to the emerging evidence that UIL-18 could serve as diagnostic index in AKI. Even though some studies did suggest UIL-18 to be a prognostic marker, since outcome was not followed in our study, it could not be commented. But there were two patients who died of AKI was found to have very high levels UIL-18 (<150 pg/ml when compared to 40 pg/ml for AKI). Whether this is just a coincidence or a causal factor needs further large scale study and statistical co-relation.

Even though, the sensitivity and specificity are convincingly high (>40 pg/ml) and the cost low for the test to be a screening tool, there are few points to be addressed before it can be considered for clinical decision making. Use of UIL18 is further complicated by its role as mediator of inflammation and its association with mortality. These lead to exclusion criteria to be many and their wide range of normal value in population. Urinary biomarkers also have a inherent limitation of being influenced by urine volumes, so their concentrations should be normalized to excretion of other solutions. (e.g. creatinine). Large prospective studies of patients in different settings will be required to ascertain the relationship of these markers to each other and to establish their capacity to predict outcomes.

UIL18 levels in pre-renal azotemia should be studied and their reference point of reversal with fluid infusion should be characterized to define specific point of intervention, inform patient decisions on choice decision, referral to higher centers for renal replacement therapy and predict outcomes.

The major delimitation of urinary IL18 comes in its utility to predict AKI in the settings of sepsis and multi-organ dysfunction (MODS). Further research is needed to qualify and quantify its reliability in those situation. This present study is aimed to provide a glimpse of future where a combination of clinical and biomarker data should provide the tools for physicians to recognize AKI, inform patients and plan further mode of treatment.

CONCLUSIONS

1. Urinary IL-18 >40pg/ml promises to be a reliable early marker of AKI compared with creatinine and urea.(with sensitivity of 87% and specificity of 84%)
2. Urinary IL-18 predates AKI by atleast 24 hours in patients developing acute kidney injury (AKI).
3. Urinary IL-18 could serve as morbidity and mortality predictor in various settings of AKI.
4. It should help physicians to recognize AKI early, inform patients, plan action, characterize the course of disease and define specific points of intervention and predict outcomes.

FURTHER RESEARCH

1. Similar studies can be carried in larger population and different setting (specially in patients undergoing cardiopulmonary bypass where renal ischemic time can be ascertained after aortic clamping).to test the exact utility of urinary IL18.
2. Relationship of different AKI markers to each other and to establish their capacity to predict outcomes.
3. Studies to test the validity of urinary IL-18 as a prognostic marker.
4. Co-relation between serum IL-18 and urinary IL-18.
5. Interleukin 18 levels in sepsis its reliability to predict multiorgan dysfunction (MODS).
6. Well designed studies to use AKI biomarkers to set standardized criteria for AKI.

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PROFORMA

NAME: AGE/SEX:

TIME OF

BITE: PLACE:

VITALS

TEMPERATURE

PULSE

BP

ANY CONDITION PREDISPOSING TO AKI

DM / HYPERTENSION / CELLULITIS / SHOCK /

CAD / SEPSIS / INFECTIONS / INFLAMMATIONS

SPECIFY IF ANY OTHER MEDICAL ILLNESS

BASELINE UREA / CREATININE

USG KUB

R	SIZE	CMD	ECHOES
L	SIZE	CMD	ECHOES

URINE OUTPUT

FOLLOW UP